Bioresource Technology 101 (2010) 2981–2987

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Isolation and quantification of cadmium removal mechanisms in batch reactors inoculated by sulphate reducing bacteria: Biosorption versus bioprecipitation

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ARTICLE INFO

Article history:
Received 29 July 2009
Received in revised form 3 December 2009
Accepted 8 December 2009
Available online 6 January 2010

Keywords:
Sulphate reducing bacteria
Bioprecipitation
Biosorption
Cadmium
Modelling

ABSTRACT

Biosorbing properties of sulphate reducing bacteria were tested to distinguish the amount of cadmium removed by bioprecipitation from that bound onto biomass surface (biosorption). Experimental results of cadmium abatement in batch growth tests (bioprecipitation tests) were then compared with metabolism-independent binding properties of SRB cell wall surface (biosorption tests performed with dead biomass). Experimental results showed that SRB inoculum removed 59 ± 5% of sulphates in 21 days even in presence of cadmium (0–36 mmol L^{-1}), while non-monotonous kinetic effects were observed for increasing Cd concentrations. Comparison between bioprecipitation and biosorption tests denoted a significant contribution of biosorption (77%) in total Cd removal (0.40 ± 0.01 mmol g^{-1}). Characterisation of bacterial acid–base surface properties by potentiometric titrations and mechanistic modelling denoted that carboxylic, phosphate and amino groups of cell wall are the main responsible of metal removal by biosorption mechanism.

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1. Introduction

Heavy metals are used in several industries, including mining, metallurgical, electronic, electroplating and metal finishing. The presence of heavy metals in final industrial effluents is extremely undesired, as they may accumulate to toxic levels and cause ecological damage under certain environmental conditions.

Many techniques have been developed for the treatment of heavy metal-bearing effluents, which can involve both abiotic and biotic methods. Abiotic methods include precipitation, adsorption, ion exchange, membrane and electrochemical technologies. These processes are often expensive, not environmental friendly, depending on the concentration of metals and producing waste sludges which must be further on treated (Costa et al., 2008; Crini, 2006).

Recently, research attention has been focused on environmentally compatible and cost effective biological methods for the treatment of effluents.

The precipitation of metals with H_2S produced by sulphate reducing bacteria (SRB) has been proposed as an alternative process for the treatment of metal-bearing effluents (Foucher et al., 2001).

SRB are anaerobes that use sulphate as the terminal electron acceptor for the metabolism of organic substrates (Postgate, 1984). The dissimilatory reduction of sulphate to sulphide (Eq. (1)) generates alkalinity and promotes metal precipitation as sulphides (Eq. (2)):

$$SO_4^{2-} + 2CH_2O + 2H^+ \rightarrow H_2S + 2H_2CO_3 \quad (1)$$

$$Me^{2+} + H_2S \rightarrow MeS\downarrow + 2H^+ \quad (2)$$

Until recently, the use of SRB was limited to *ex situ* treatment in sulphidogenic bioreactors (Gonçalves et al., 2007), but latterly attention has focused on their application in *in situ* passive systems, such as artificial wetlands and, more recently, permeable reactive barriers (PRB) (Costa et al., 2008; Jarvis et al., 2006).

Full-scale applications of biological PRB are generally characterised by the use of solid organic mixtures as electron donor for the sulphate-reduction (Cruz Viggi et al., 2010; Pagnanelli et al., 2009; Costa et al., 2008; Foucher et al., 2001; Jarvis et al., 2006).

Both in PRB and sulphidogenic bioreactors, a wide range of reactions takes place which can remove metallic contaminants from water. These include bioprecipitation (biologically-mediated precipitation of metal as sulphide), chemical precipitation, adsorption onto inorganic components of PRB filling, biosorption onto organic materials used as substrates for biomass growth, and biosorption onto SRB surface. This last mechanism acquires great importance in sulphidogenic bioreactors, but it should be also taken in account for PRB design.

In fact biosorbing properties of bacterial biomasses are widely reported in the literature (Beolchini et al., 2003; Esposito et al., 2001; Pagnanelli et al., 2000; Uslu and Tanyol, 2006; Vijayaraghavan and Yun, 2008; Volesky, 2007). Cell wall composition is one of

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doi:10.1016/j.biortech.2009.12.009

the most important factors affecting bacterial biosorbing properties. Two general types of bacteria exist, Gram-positive and Gram-negative; their cell wall composition is quite different. The anionic functional groups present in the peptidoglycan and teichoic acids of Gram-positive bacteria (Sonnenfeld et al., 1985), and the peptidoglycan, phospholipids and lipopolysaccharides of Gram-negative bacteria (Beveridge, 1999) were the main responsible for the anionic character and then metal-binding capability of the cell wall. Extracellular polysaccharides are also capable of binding metals, but their availability depends on the bacterial species and growth conditions (McLean et al., 1992; Yee and Fein, 2001).

As for SRB, they are a complex physiological bacterial group; they can be both Gram-positive and Gram-negative (Castro et al., 2000). The biosorption capacity of *Desulfovibrio* species (a Gram-negative genus of SRB) was investigated in the removal of aluminium, zinc, copper, platinum and palladium (de Vargas et al., 2004; Chen et al., 2000; Hard et al., 1999). These works involved biosorption studies conducted with metals and operative conditions (e.g. pH) different from those investigated in this study. However, these studies gave results comparable with those reported in this manuscript, in terms of SRB maximum sorption capacity.

Regarding the treatment of metal-bearing solutions exploiting SRB activity, most of literature papers lack for the isolation of the different mechanisms operating in metal removal. In this view, this paper describes batch growth experiments (bioprecipitation tests performed at different concentrations of metal), compared with metabolism-independent binding properties of SRB surface (biosorption tests performed with dead biomass in different operating conditions), for the isolation and quantification of removal mechanisms of cadmium by a SRB consortium. Discrimination between the different mechanisms is necessary in order to avoid an overestimation of the sulphate-bioreduction capacity of the system, and then misleading results in the following scale-up and design phases.

2. Methods

2.1. SRB maintenance and growth

SRB inoculum was kindly furnished by the research group of Professor Groudev (Department of Engineering Geoecology, University of Mining and Geology, Sofia, Bulgaria), who collected it in the Curilo mine district located near Sophia (Groudev et al., 2001).

Bacteria used in the experiments were cultivated in closed shaken flasks using standard procedures for SRB reported in the literature (Postgate, 1984). C Medium, used for bacterial growth and acclimatizing, has the following composition ($g\,L^{-1}$): KH_2PO_4 (0.5), NH_4Cl (1), Na_2SO_4 (4.5), $CaCl_2{\cdot}6H_2O$ (0.06), $MgSO_4{\cdot}7H_2O$ (0.06), sodium lactate (6), yeast extract (1), $FeSO_4{\cdot}7H_2O$ (0.004), Na_2S (1) and sodium citrate (0.3).

Glass reaction flasks (120 mL), containing a sampling port, were used for all the experiments. The 80 mL of C Medium were added in flasks; therefore the flasks were sealed and 20 mL inoculum of bacteria (inoculum volume was 20% of total volume) cultivated in C Medium (in exponential growth phase on the 7th day) were added by a sterile syringe through the sampling port. All experiments were conducted at room temperature under shaking conditions.

The pH, E_h, SO_4^{2-} concentration and H_2S production were monitored during the growth. Measurements of pH (by CRISON GLP22), E_h (by CRISON GLP22) and H_2S (by lead acetate paper) were determined immediately after sample collection. Samples were then filtered through 0.45 μm cellulose acetate filters and used for sulphate determination (Cruz Viggi et al., 2009).

Medium was regularly sampled for the determination of biomass concentration, as volatile suspended solids (VSS), according to standard methods (APAT, 2003).

2.2. Batch growth tests with cadmium

Flasks were prepared as those described in the Section 2.1, but adding cadmium (from a stock solution of 1000 ppm of Cd in nitric acid) in order to have different initial concentrations of metal. Three different kinds of tests were carried out using the same inoculum in order to avoid effects due to inocula variability: M_1 (no Cd), M_2 (0.18 mmol L^{-1} of Cd) and M_3 (0.36 mmol L^{-1} of Cd). Each test was performed twice and average values were considered. The pH, E_h, H_2S production, SO_4^{2-} and cadmium concentrations were monitored during the experiments. Cadmium concentration was determined by an inductively coupled plasma spectrophotometer (ICP).

2.3. Bioprecipitation tests

Samples for bioprecipitation tests were prepared as those described in the Section 2.1, but adding metal spikes during SRB growth. A metal ion stock solution was prepared dissolving nitrate salt in distilled water. Cd was added, from stock solution, in order to have an increase of metal concentration of 0.36 mmol L^{-1} for each addition. Three days after each addition a sample was collected (3 mL), and the same volume of metal-bearing solution was added to the flask, maintaining a constant total volume.

Blank tests using C Medium without SRB inoculum were also performed in order to distinguish the amount of Cd (C_i) removed by biological mechanisms (bioprecipitation and biosorption) from that (C_b) removed by chemical precipitation as CdS due to the presence of Na_2S in cultivation medium.

The pH, E_h, S^{2-} production, SO_4^{2-} and Cd concentrations were monitored during the experiments. Each test was performed twice and average values reported.

2.4. Biosorption tests

Preliminary biosorption tests were performed using biomass samples with different age (5, 10 and 15 days). Biomass samples were prepared as described in Section 2.1. After 5, 10 and 15 days, 40 mL samples of biomass suspension were aerated overnight to kill anaerobic bacteria and then put in contact with 0.09 mmol L^{-1} Cd solution. Metal removal was investigated at pH 7 (adjusted with HNO_3 or NaOH additions). Metal-bearing suspensions were kept under magnetic stirring at constant pH until the equilibrium conditions were reached after 2 h. Solid–liquid separation was performed by centrifugation (5 min at 4000 rpm) and equilibrium cadmium concentration in liquid phase was determined by ICP. For each condition blank tests without biomass were also performed to determine the initial metal concentration.

Biosorption isotherms were carried out using 5-day biomass samples and 40 mL of Cd solution (with the initial concentration C_0 in the range of 0.09–0.45 mmol L^{-1}). Metal removal was investigated at three different pH levels (7.0, 7.5 and 8.0) by adjustment with HNO_3 or NaOH additions. Final metal concentration (C_{eq}) was determined by ICP.

2.5. Potentiometric titrations

Potentiometric titrations were performed using aerated suspensions of biomass at 5 days (40 mL).

Suspensions were fluxed by N_2 to remove CO_2, and titrated by standard solutions of NaOH 0.1 N (basic branch) and HCl 0.1 N (acid branch) (Pagnanelli et al., 2004). After each addition of titrant

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